

A newly observed form of symbiotic relationship between Reverdin's blue *Lycaeides argyrognomon praeterinsularis* (Verity), (Lycaenidae) and *Camponotus japonicus* Mayr (Formicidae)

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Abstract. Observations of an individual larva of Reverdin's blue named O-I, and its tending ants were made in the grassland at the foot of Mt. Fuji, Japan between 29 VIII to 1 X in 2003. During its 3rd to 4th instars, O-I larva was almost entirely protected from natural enemies by *Camponotus japonicus* Primary Tending Ants (PTAs) and Secondary Tending Ants (STAs). Prior to pupation, O-I entered a *C. japonicus* nest hole with a PTA and three unmarked ants. Many *C. japonicus* individuals, including the same PTAs and some STAs from the larval stage, as well as newly attending STAs, alternatively accompanied the pupa until emergence. This is a new form of symbiotic relationship between Reverdin's blue and *C. japonicus*. In the relationship characterized by what we term "loyalty," a specific individual of Reverdin's blue was tended by one or several PTAs, and some STAs, from the 2nd instar to adulthood.

Key words: facultative or obligate ant-association, Reverdin's blue *Lycaeides argyrognomon*, *Camponotus japonicus*, new form of symbiotic relationship, 'loyalty' system.

INTRODUCTION AND METHODS

The study area is situated at 35° 26' N 138° 49' E, at 1020 m altitude and extending horizontally 11.6 km northeast from the summit of Mt. Fuji (Fig. 1). The surrounding area is part of the North Fuji training ground for Japan's Self-Defense Force. The landscape is a savannah of grassland (*Miscanthus sinensis*) with patchily distributed pine forests (*Pinus densiflora*) or shrubs (*Salix integra*).

The survey was carried out from 12 VII to 26 X in 2003 within an area of 14 m², including an isolated colony of the *Indigofera pseudotinctoria* (Fig. 2). Total observation time was 7652 minutes. The time spent observing the Reverdin's blue individual, O-I was 6399 minutes, extending over 27 days from 25 VIII to 1 X. Table 1 summarizes the data taken over 30 minute-long observation periods from 29 VIII to 1 X (total 5077 minutes).

We marked the butterfly larva and tending *C. japonicus* ants with colored felt-tipped pen in order to distinguish them individually. The marking seemed not to disturb O-

I larva or tending ants because the larva quickly resumed its usual activities and the ants returned to tending O-I after cleaning their bodies to remove the new scent. After marking, appeared the liquid to quickly evaporate, leaving only dry marks on the subjects.

Reverdin's blue larvae have a DNO (Dorsal nectary organ) in the 7th segment. This study was conducted on the supposition that ants were attracted to secretions from the DNO's. We used video and digital cameras at a distance so as not to disturb the larva and the ants.

We mainly recorded behavior of individual tending ants and the time spent engaged in these behaviors and the responses of O-I being tapped by the tending ants' antennae.

On 23 IX, when I picked up a stone attached to the O-I pupa in its pupal stage, the attending ant cut the girdle yarn of the pupa. I bonded the end of the abdomen of O-I pupa to the attached rock and made girdle yarn with Cemedine glue. These treatments may have affected the time spent by O-I emerging from its pupal case.

RESULTS

Table 1 summarizes the events for O-I (marked

Table1. The individual changes and the percentage of ants tending to Reverdin's blue O-1 from 2nd instar to adulthood. PTA (STA) indicates Primary (Secondary) Tending Ant and 1-7 numbers indicate individuals. Cjs or Fjs indicate ant individuals of *Camponotus japonicus* or *Formica japonica* not individually marked. Percentages in each block illustrate the proportion of daily observation time each ant individual tended O-1. 'None' indicates the percentage of the time no ants tended O-1.

Stage of Reverdin's blue	Larva (2nd)	Larva (3rd)	Larva (4th)	Larva (4th)	Larva (4th)	Larva (4th)	Larva (4th)	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa-adult	Adult	Observed/Total (days)
Individual/Date	Aug 29	Sep 1	Sep 4	Sep 5	Sep 6	Sep 7	Sep 10	Sep 13	Sep 15	Sep 16	Sep 18	Sep 19	Sep 20	Sep 22	Sep 26	Sep 27	Sep 28	Sep 30	Oct 1	Observed/Total (days)
PTA-1	69,6 %	xDied																		1/19
PTA-2		88,5 %	•	•	29,0 %	78,6 %	•	•	0,5 %			0,7 %	0,6 %		•	7,6 %	•	•	25,4 %	15/19
PTA-3			•	•	92,5 %	81,2 %	•									•				6/19
STA-1	8,5 %	8,3 %										•	•							4/19
STA-2	13,5 %	0,1 %																		2/19
STA-3												•	•	•						6/19
STA-4														•		•	•			4/19
STA-5													•	•		•	•	•	•	6/19
STA-6													•	•						2/19
STA-7														•	•	•	•	•		5/19
Cjs	1,3 %						•	•	•				•	•		•				7/19
Fjs	2,3 %	0,1 %																		2/19
None	11,6 %	3,1 %			0,2 %	0,2 %														Total minutes
Observation time (minutes)	512	337	55	78	407	810	45	30	536	41	40	587	476	60	48	531	37	312	135	5077

with an orange spot from the 2nd through 4th instars) and the three PTAs and seven STAs of *C. japonicus* attending from the 2nd instar until the emergence of the butterfly.

During the 2nd instar, on 29 VIII, PTA-1 dominated attendance (present 69.6 % of the time; 63% of the time solely, 6.6 % of the time with STAs). During the 3rd instar on 1 IX, PTA-1 died and was carried off by a *Formica japonica* ant to its nest. O-1 was then attended by a new ant, PTA-2, a nest mate of PTA-1's. On this day PTA-2 dominated attendance of O-1 (88.5% of the time). During the 2nd to 3rd instars, a few unmarked *C. japonicus* or *F. japonica* ants tried to attend O-1 larva for short time intervals in the absence of PTA-1 or PTA-2.

From 4 to 7 IX, PTA-2 and PTA-3 wholly tended the 4th instar O-1. During the periods when only one ant was present either, PTA-2 or PTA-3, that individual was observed to tap the posterior part of O-1 larva near the DNO. When both attended together, they tapped different parts of O-1: PTA-3 the front part and PTA-2 the rear part near the DNO (Fig. 3-A), respectively. As the total time PTA-2 spent in attendance around the DNO was longer than that spent by PTA-3, PTA-2 took precedence over PTA-3. During the observation period, the time that no ant attended O-1 decreased from 12% in the 2nd instar to 3% in the 3rd and then to 0.2% in the 4th (Table 1, 29 VIII to 7 IX). O-1 was almost entirely protected from natural enemies during the period of 3rd to 4th instars by *C. japonicus*.

Because O-1 could not be found on the host plant on 10 IX, a stone (ca. 5 cm radius) was lifted near the host plants. Subsequently O-1 was found under the stone with an unmarked ant. Instead, O-1 was in a "room" formed by small pebbles; PTA-2 and two unmarked ants were there. O-1 larva then started to crawl under another small stone (ca. 1 cm in radius situated at the entrance of a *C. japonicus* nest hole) with PTA-2 and the three unmarked ants (Fig. 3-B). PTA-2 was searching and carrying a small pebble (2-3 mm in radius) from the original "room" to the nest hole occupied by O-1 larva (indicated by yellow arrows in Fig. 3-B), while other unmarked ants carried away many small pebbles surrounding O-1 larva. O-1 changed to pre-pupa the next day and pupated three days later.

The time ants spent under the stone during the pupal period was assumed to be the same as time spent tending to the O-1 pupa. That of PTA-2 turned out to be 0.5-0.7% of the total observed time (five to six minutes) for the six days following pupation, drastically less than that the time spent during the larval stage (Table 1). Table 1 shows that many individuals, including the same PTAs and STAs from

the larval stage and newly attending STAs, took turns tending the pupa. During the three days before butterfly emergence, PTA-2 spent much more time (7.6% of the total) under the stone and more than seven ants tended to the O-1 pupa.

O-1 began to emerge at 14:08 on 30 IX; 190 minutes later PTA-2 approached the emerging O-1 imago and began tapping the head and body with its antennae, and continued to do this for ten minutes until O-1 had partially extracted itself from the pupal case (Fig. 3-C).

PTA-2 then left the adult female O-1 without attacking (Fig. 3-D). Subsequently STA-7, which had attended the O-1 pupa, approached the emerged adult as it was hanging from a grass blade (about three cm height from the ground) and gently tapped it for about an hour (Fig. 3-E). During this time, PTA-2 tried to remove O-1's pupal case.

When the adult fell to the ground, PTA-2 followed. After the O-1 adult climbed up another grass blade, PTA-2 continued to walk on the ground for the remaining period of observation (80 mins) within about a meter radius of the grass blade but without climbing it. The observations of 30 IX are described in the Appendix. On 1 X, following emergence, we found the O-1 adult on the ground, having failed to expand her wings. STA-5, which had attended the O-1 pupa, was tapping gently on the O-1 adult with its antennae (9:30; Fig. 3-F). PTA-2 spent 25.4 % of 135 minutes removing O-1's pupal case.

DISCUSSION

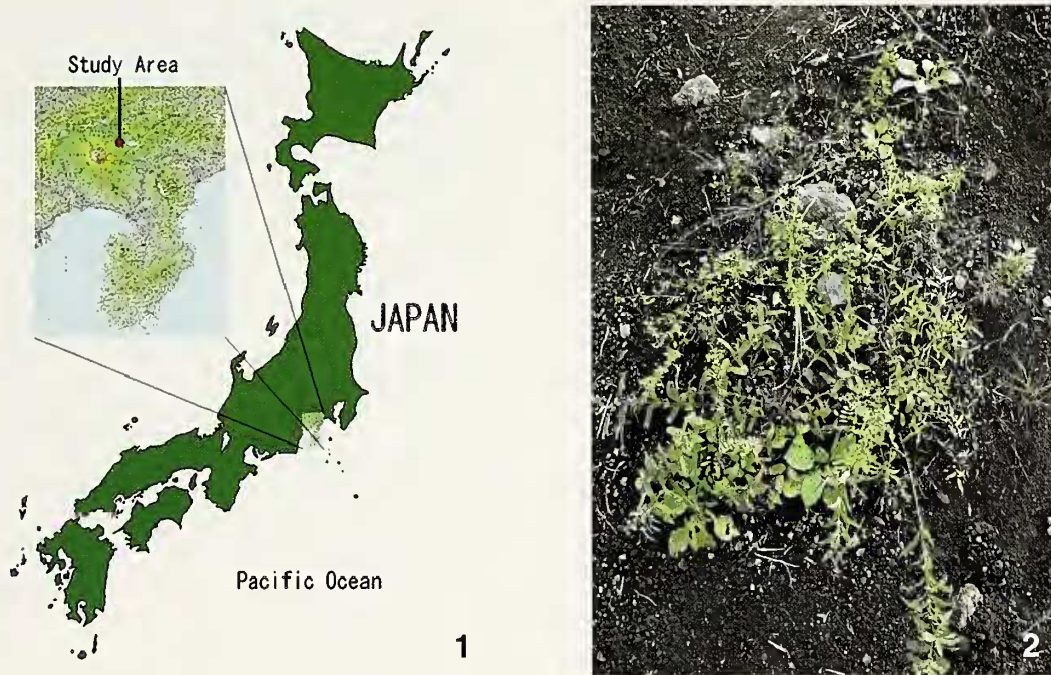
Mutualism is a successful adaptive strategy for butterflies (Hölldobler & Wilson, 1990). In Lycaenidae, with more than 6000 species worldwide, larvae of the majority of species studied maintain some association with ants at least during later stages of development (Fiedler, 2001).

Lycaenid-ant interactions have been classified with two types of ant-association: obligate, meaning larvae are dependent on ants for survival; or facultative, meaning larvae do not require attendant ants for survival (Bronstein, 1994; Osborn & Jaffe, 1997; Pierce *et al.*, 2002; Heath & Claassens, 2003).

Obligate myrmecophiles were classified further into subgroups obligate mutualist and obligate parasite (Fiedler, 2001), believed to be the result of chemical mimicry (Akino *et al.*, 1999; Thomas & Settle, 2004; Als *et al.*, 2004).

Reverdin's blue is a facultative species because it can be reared from egg to adult without any ants in captivity.

We do not know of any paper on symbiotic



Figures 1-2. 1. Study area. 2. Study site

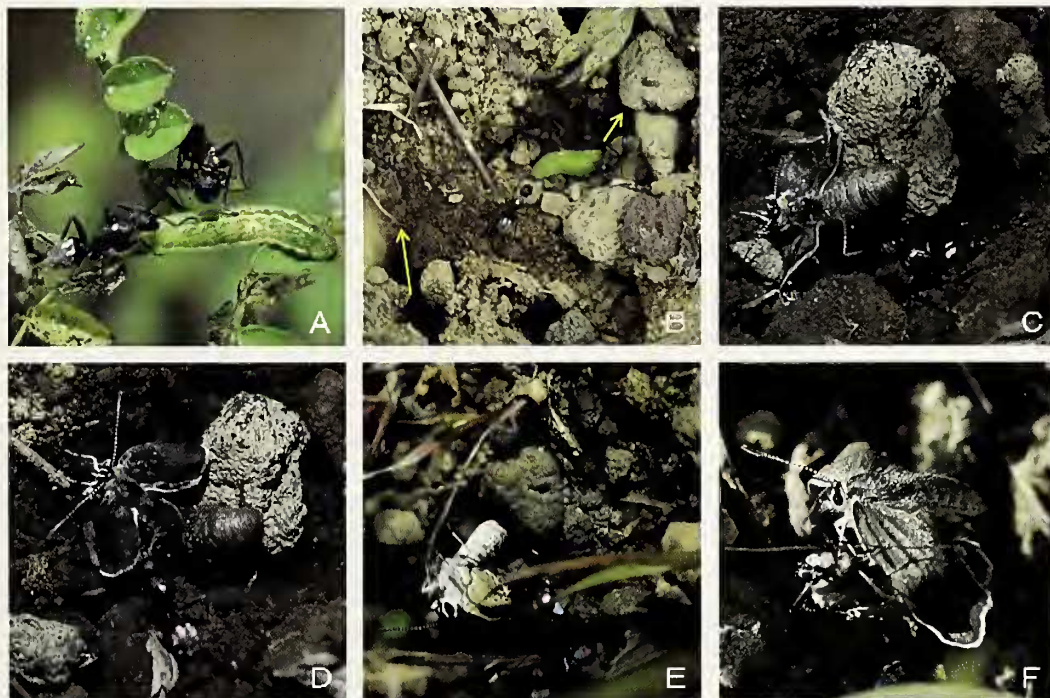


Figure 3. The "loyalty" system in the newly discovered symbiotic relationship between Reverdin's blue and *Camponotus japonicus*. **A:** Two Primary Tending Ants (PTA-2, left and PTA-3, right) tending to larva O-1 (in 4th instar). **B:** Movement of the larva O-1 from the original pupation room (left arrow) to a new one (right arrow) at the entrance of ant nest with PTA-2 and three unmarked *Camponotus* ants. **C:** Tending behavior of PTA-2 toward the emerging pupa of O-1. **D:** Emerged imago in process of being tapped by PTA-2. **E:** Tapping behavior of Secondary Tending Ant (STA-7) aimed at hanging O-1 adult. **F:** Tapping behavior of STA-5 aimed at O-1 adult on morning following emergence (All photos taken by Watanabe).

behaviors, whether obligate or facultative, that has individually identified a butterfly and its tending ants. This paper has focused on individual behavior in the symbiotic relationship between the butterfly and its tending *Camponotus* ants during the period from larva to adult in nature.

Reverdin's blue had been previously reported to pupate on the larval host plant by using leaves to make its own nest (Fukuda *et al.*, 1984). In our study the last instar larva enters the ant's nest hole by its own effort, although accompanied by PTAs and STAs, to pupate there. Over forty Reverdin's blue pupae were found in or near ants' nest holes in 2004 and 2005 (unpublished data).

Our study also shows PTAs and STAs of *C. japonicus* tend Reverdin's blue's pupae until emergence. In our case of O-1, the tending *C. japonicus* concentrated on limited sections of the surface at the rear of the head and the abdomen of the O-1 pupa. Moreover, O-1 attracted at least nine individuals of the same colony to its nest throughout the pupal stage (Table 1).

In many myrmecophilous species, emerging adults are often killed if they fail to escape (Pierce *et al.*, 2002). So why was the emerged adult O-1, not attacked by its tending PTA, STAs, or other resident ants even when it took about ten minutes to emerge from the pupal case failed to expand its wings for over 2 hours.

C. japonicus PTAs thus appear to display a form of "loyalty" toward Reverdin's blue across all stages from larva to adult. This is a newly identified case of symbiotic relationship.

These observations concern just one individual of Reverdin's blue, O-1. However, the finding is supported by many other observations from 2004 to 2007 (unpublished data). During these four years of observations, the PTAs tending a particular specimen of Reverdin's blue sometimes turned over, but in small cases, specific PTAs or STAs of *C. japonicus* continuously tended one specimen as it developed from the 2nd instar to the pupal stage. The summary evidence clearly supports symbiotic "loyalty" by *C. japonicus* toward Reverdin's blue.

Reverdin's blue may have similar symbiotic relationships with at least four species of tending ants, *C. japonicus*, *F. japonica*, *F. yessensis*, and *Lasius japonicus* (unpublished data 2004-2005).

As symbiosis between butterfly and ants is an important interspecific interaction of ants with Lycaenids (Fiedler, 1998 and 2001; Pierce *et al.*, 2002; Heath & Claassens, 2003) and are characterized by a high degree of ecological opportunism and flexibility in associating with ants (Fiedler, 2001), the observed individual symbiosis described here may be

relatively common among other species pairs. Further observations and experiments will be conducted to better understand this symbiosis, particularly with endangered Lycaenid butterfly species. The relationship could be a key to conservation of endangered Lycaenid populations.

ACKNOWLEDGEMENTS

We thank volunteer members of Earth Watch Japan, as well as Mr. R. Nakajima and Miss K. Sakamoto, for their support in conducting field surveys. Moreover, we thank Dr. R. L. H. Dennis, Mr. M. Takahashi, Dr. K. Honda, Dr. A. Bernstein, Dr. B. Machin and Mr. K. Sei for suggestions regarding our observation data, the early draft of this article, or the reviewed manuscript, as well as the staff of the North Mt. Fuji training ground of the Japan Self-Defense Force for granting us permission to conduct our study in the training area. We also thank Dr. Konrad Fiedler for the suggestive and helpful review to our manuscripts and Dr. Rudolf Mattoni for the useful editing.

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APPENDIX

Detailed observations of emergence of O-1 on 30 IX. It was the first and longest observation of the Reverdin's blue made in the survey.

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|-----------------|--|
| 14:08 | It started emerging. A small crack on the pupa was observed with a camera with a close-up lens. |
| 15:14 | The crack got larger; it could be seen with the naked eye. The ants carried small pebbles around the pupa, and the stone with the pupa attached was repositioned to face us. |
| 17:17 | PTA-2 approached the emerging pupa and tapped on the body of both the pupa and the adult with its antennae (Fig.3-A). This behavior continued to 17:27. |
| 17:27 | The female O-1 adult emerged from the pupa (Fig.3-B). PTA-2 attended O-1 as it walked up the slope of the nest, tapping it once with its antennae. PTA-2 did not attacked to O-1 walking up slowly in front of PTA-2. |
| 17:30 | As O-1 hung from the top of a blade of grass (about 3cm high), STA-7 approached O-1 and began tapping the body with her antennae (Fig.3-C). This behavior continued until 18:29, as PTA-2 tried to take off the shell of the pupa (Fig.3-D). |
| 18:29 | PTA-2 approached O-1 and came into contact with STA-7. |
| 18:42 | When O-1 moved to a higher point (about 5cm from the ground), STA-7 disappeared after contacting PTA-2. O-1 remained still while PTA-2 wandered around O-1 or tried to remove the shell of the pupa. PTA-2 never tried to climb up to O-1. |
| 19:30:48 | O-1 moved to another point (about 10 cm high) and continued to hang on. When Watanabe tried to mark the legs of O-1 with a blue felt-tipped pen, O-1 moved its unstretched wings. |
| 19:30:58 | When Watanabe tried to mark O-1 again with the felt-tipped pen, O-1 dropped down to the ground. Just then, PTA-2, which had been taking off the shell of the pupa, approached O-1. PTA-2 looked for O-1 on the ground. |
| 19:32:21 | PTA-2 found O-1 walking on the ground and tapped on O-1 with its antennae. PTA-2 followed O-1 and induced O-1 to climb up to a higher point with its antennae-tapping. |
| 19:40:30 | PTA-2 and O-1 separated from each other. |
| 19:41:50 | O-1 climbed up the grass 10cm high from the ground and remained still. We observed O-1 and PTA-2 until 21:00. O-1 rested at the same point while PTA-2 was wandering on the ground around O-1 within about a one-meter radius. |
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